Methylation Analysis and Whole Genome Chromosomal Microarray for Angelman Syndrome

Clinical Features

Angelman syndrome (AS) is a neurological disorder affecting development and behavior. Individuals with Angelman syndrome exhibit developmental and cognitive delays typically noted in the first year of life, including absent or significantly impaired speech. Neurological features include seizures, ataxia, and characteristic electroencephalogram (EEG) abnormalities. Characteristic behavioral features include sleep disorders and a happy demeanor with recurrent laughter, smiling, and excitability. Individuals with AS are typically noted to have prominent chin, small head circumference and a wide mouth with protruding tongue. The presence and severity of the clinical features can vary among individuals with AS.[1]

Genetics

AS is an imprinting disorder caused by one of four known mechanisms that result in absence of expression of the *UBE3A* gene on the maternally derived chromosome 15 within 15q11.2- q13.1. The majority (65-75%) of patients with AS have a large recurrent microdeletion extending from 15q11.2 to 15q13.1 on the maternally inherited chromosome.[2] Paternal uniparental disomy (UPD) accounts for 3-7% of patients with AS.[2] Approximately 3% of patients have an imprinting error that establishes a paternal chromosome-specific methylation pattern despite the presence of both parental alleles, and these imprinting errors can be caused by a microdeletion within the imprinting center in 15q11.2 (0.3% of all AS cases) or by an unknown mechanism that inappropriately silences genes regardless of the parental origin of the chromosome (2.5-3% of all AS cases).[1,2,4] *UBE3A* variants detectable by sequencing are responsible for 5-11% of AS cases, while rare patients have been reported to harbor a partial deletion of the *UBE3A* gene.[2] The etiology of the remaining AS cases (~10%) is unknown.

The majority of cases of genetically confirmed AS are *de novo* with a recurrence risk of <1%; however, the recurrence is 50% for an inherited imprinting center deletion, a maternally inherited *UBE3A* variant or partial deletion, or for microdeletions inherited from a mother with a balanced chromosome rearrangement.[2] Patients with Angelman syndrome who have large deletions have the most severe phenotype, with severe seizures, cognitive delays and an absence of speech.[3] Patients with AS due to paternal UPD and imprinting errors tend to have a less severe presentation, with a lower occurrence of seizures and better cognitive and speech development. .

Test Methods and Sensitivity

Test	Abnormalities Identified	Detection Rate	Comments
Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA)	- common 15q11.2-15q13.1 deletion - methylation abnormalities due to paternal UPD and imprinting errors	~80% AS	can confirm AS diagnosis and determine if due to deletion or abnormal methylation; test cannot distinguish between

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			paternal UPD and imprinting errors
Whole Genome Chromosomal Microarray (CMA)	- common 15q11.2-15q13.1 deletion - paternal UPD	~70-75% AS	can confirm AS diagnosis and determine if due to deletion or paternal UPD

Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) can detect the common 15q11.2-15q13.1 deletion and determine the parent-specific methylation imprint. An abnormal MS-MLPA result can confirm a diagnosis of AS and reveal the mechanism (deletion or UPD/imprinting error; but cannot distinguish between UPD or an imprinting error). Methylation analysis is available as a first-line test for patients with a suspected diagnosis of AS or can be performed as reflex testing following normal array results for individuals with a high suspicion of AS. This test does not offer imprinting center sequence analysis. However, patients with abnormal methylation studies but normal whole genome SNP array likely have an imprinting error, if uniparental heterodisomy has been ruled out. Using genomic DNA from the submitted specimen, Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) is performed to determine the copy number of the evaluated gene(s) in this specimen compared to control specimen(s), and the imprinting status of the region of interest. This quantitative test includes simultaneous PCR amplification, electrophoretic separation and fluorescence detection of PCR amplicons from the evaluated gene(s) along with internal standards.

Whole genome chromosomal microarray (CMA) can detect the common 15q11.2-15q13.1 deletion and paternal UPD; however, CMA cannot identify pure uniparental heterodisomy (i.e., can only identify uniparental isodisomy, mixed hetero- and isodisomy, or segmental isodisomy). Imprinting center deletions (but not other types of imprinting errors) and intragenic deletions or duplications of the *UBE3A* gene that meet the reporting threshold for this array may also be detected. Whole genome CMA is performed using the Affymetrix CytoScan HD microarray system. The array contains 2.67 million probes placed throughout the genome that are spaced on average 880 bases apart in genic regions and approximately 1,700 bases apart in non-genic regions. There are 1.9 million non-polymorphic probes for detection of copy number variants (CNVs) and 750,000 single nucleotide polymorphism (SNP) probes. The array can identify deletions ≥25 kb including at least 25 consecutive probes and duplications ≥50 kb including at least 50 consecutive probes, and can therefore identify other genomic imbalances that may produce an AS-like phenotype (e.g. 2q23 or 17q21.31 deletions). In addition, the array also identifies regions of homozygosity (ROH), including uniparental disomy (UPD) and identity by descent (parental consanguinity) on all autosomes. Autosomal ROH is reported when at least one region of homozygosity of ≥10 Mb or two regions that are each ≥8 Mb are identified. Any additional ROH calls ≥5 Mb are included in the report.

References

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